The Viral Nucleic Acids formed in Chick-Embryo Cells Infected with Semliki Forest Virus

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When chick-embryo cells, previously infected with Semliki Forest virus, were treated with actinomycin and then pulsed with [³H]uridine before extraction of the nucleic acids with phenol plus sodium dodecyl sulphate, three species of RNA were found. These were single-stranded viral RNA, sedimenting at 45s in sucrose density gradients, and a 26s single-stranded species and a 20s double-stranded species, which were only partially resolvable on the gradients (Friedman, Levy & Carter, 1966; Son nabend, Martin & Mecs, 1967).

The three species were separable by electrophoresis in 2.5% polyacrylamide gels by using the system of Peacock & Dingman (1967), or by sequential extraction of the infected cells with phenol and then with phenol plus sodium dodecyl sulphate. Phenol extracted only the 20s double-stranded viral RNA, which was further purified by treatment with 4M-lithium chloride, to give a product that was about 75% resistant to ribonuclease (1 µg./ml. for 10 min. in 2 × SSC buffer), had a density in caesium sulphate of 1.635 g./cm.³ and a thermal transition temperature of 107°, and gave a single band on polyacrylamide-gel electrophoresis with a mobility intermediate between that of the 28s and the 16s ribosomal markers. After the phenol extraction, the 26s and 45s species were extracted with phenol plus sodium dodecyl sulphate. Both species had a density of 1.680 g./cm.³, were completely degraded by ribonuclease, and were readily separable by polyacrylamide-gel electrophoresis, the 45s species having a very low mobility and the 26s species a mobility slightly less than that of the 28s ribosomal marker. Attempts to demonstrate transfer of radioactivity from 20s RNA to 26s or 45s RNA by pulse-chase techniques were unsuccessful, but both ³H-labelled 26s and 45s RNA displaced radioactivity from ¹⁴C-labelled 20s RNA on heating to 110° and cooling. Short pulses showed that 26s RNA was the first single-stranded species formed. Comparison of the sedimentation velocities and electrophoretic mobilities of the 26s and 45s species, together with the sharp thermal transition of 45s to 26s at 60°, suggest that 45s RNA comprises two 26s RNA species, rather than these being different conformational states of the same molecule as suggested by Sreevalsan, Lockart, Dodson & Hartman (1968).

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The Use of Polyethylenesulphonate in the Fractionation of Proteins and some Aspects of its Application to the Separation of Chromatin Proteins

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Recent interest in this laboratory has centred on the biological importance of the non-histone proteins of chromatin (Paul & Gilmour, 1968; Gilmour & Paul, 1969) and a number of methods for their isolation are currently being developed (MacGillivray, Ingles & Krauze, 1969).

Chromatin proteins can be obtained in high yield in the supernatant after ultracentrifugation of chromatin dissociated in 2M-NaCl-5M-urea (Bekhor, Kung & Bonner, 1969). Since removal of the salt and urea from this supernatant causes precipitation of the proteins, we considered that the presence of the soluble polyanion polyethylenesulphonate (PES) during this process should lead to the selective precipitation of the histones, leaving the non-histone proteins in solution.

In model systems PES was found to be an effective precipitant of histones, maximum precipitation being dependent on the pH, urea concentration and the PES/histone ratio. However, at ionic strengths up to 0.1, maximum precipitation was independent of these parameters. Under somewhat different conditions of pH etc., serum albumin and globulin could also be precipitated with PES. Surprisingly, at low ionic strengths both albumin and globulin were maximally precipitated below pH 4, but PES could distinguish between these proteins, since on increasing the ionic strength globulin was precipitated at a more acidic pH. The ability of PES to fractionate a group of proteins was confirmed by using a mixture of histones, albumin and globulin. Histones were precipitated at pH 7.5 and 1.0-0.5, and then the ionic strength was increased to 0.4. Albumin was precipitated at pH 3.8-3.45 and globulin subsequently precipitated at pH 3.0.

Ultracentrifuge supernatants from calf thymus chromatin were concentrated tenfold by ultrafiltration. For the precipitation of histones the salt and urea concentrations were lowered to 0.1M and 0.25M respectively in the presence of PES at